Welcome to Project Helio!

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Project Overview

Mass micronutrient deficiency is invisible. In talking to a dozen data collectors from seven nonprofits, we realized this invisibility makes it challenging for them to know where to establish fortification/supplementation interventions. As a result, they must collect thousands of blood samples and perform lab tests.

In Nigeria, it is estimated that 32% of the population has severe micronutrient deficiency, however 80% of them are not receiving treatment. Data on at-risk populations (women +children) is being collected on a 20-40 year basis, and consequently, micronutrient deficiency continues to remain invisible. The high costs of shipping blood samples overseas , and the lab tests ($15-60 USD per biomarker) themselves are the main barrier to collecting more actionable data (>$10M total).

In enabling in-country assessment and reducing the cost per assay to <$5 USD with a low-cost biosensor, national surveys could reach $250,000, allowing actionable data to be collected every 2 years. This cost would be achieved via leveraging common resources like paper (remodeling the pregnancy test) and off-the-shelf glucometers.

First, we plan to target vitamin B-12, since it is the only micronutrient of public health concern (there are six) that does not yet have a low-cost assay developed specifically for low-resource settings that measures holoTC, its recommended biomarker. It also has the fewest number of programs established, both in Nigeria and across the majority of LMICs. So far the project has support from mentors at Harvard, MIT, The Knowledge Society, and cluB-12 UK, a group of B-12 scientists.

Why the Project Should be Funded

Why this project should be funded

Why this project matters
In low-middle income countries, mass micronutrient (MN) deficiency is invisible, making it challenging for nonprofits/governments to establish fortification/supplementation

programs. Consequently, they must collect masses of blood samples on a population and analyze them in a lab.

Vitamin B-12 is known to have equally dangerous effects as iron and vitamin A deficiency, damaging the central nervous system (causing loss of limb function and vision). In most LMICs, ⅓ of the population is estimated to be severely B-12 deficient. Yet B-12 remains the most underrated MN among the six MNs of public health concern. Since 1980, only 5.1% of LMICs have had population B-12 data collected, resulting in the fewest established vitamin B-12 programs compared to the other six MNs. In contrast, over 55% and 40% of LMICs have vitamin A and iron data, making vitamin A and iron programs the most common.

The main barrier of collecting data is the high cost of shipping blood samples overseas ($10 M to assess the status of Nigeria). We will reduce this cost by 40x by designing a low-cost POC biosensor suitable for use in low-income areas.

Why we are ready for funding

We are at the stage where we need to build a lab prototype. We have a very thorough map of the biosensor design (components + how it will work), receiving positive feedback from over ten professors, post-docs, and grad students who also work in the low-cost diagnostic space. The building protocol will be finished in the coming month, and we have calculated that the biosensor should be able to detect concentrations of our target biomarker accurately within 2.5 minutes. WHO and CDC have shown interest, but to get to the next level we need to have a tangible device. There are shortcomings we can only find if we build a physical device, which we will be ready to do this fall. This would be impossible without funding.

Questions from Round 1 (summary of project)

Activities involved in project

Building a paper-based POC biosensor for population assessment of vitamin B-12 deficiency in LMICs.

Populations that will benefit

Nigeria, whereby populations at risk (children + women) have not had vitamin B-12 data collected on them within the last 20 years even though 31% of the population is estimated to be severely deficient. They should be evaluated every 5 years, however, this has not been made possible due to the high costs of data collection ($10M). As a result, there are no current reports of large-scale intervention programs. We have chosen Nigeria as a suitable country, but eventually it will benefit populations in all LMICs.

Outcomes of project

This project will lead nonprofits/governments to establish further and more effective population-based intervention programs (fortification/supplementation) for vitamin B-12. Mass B-12 deficiency is invisible, making it challenging for nonprofits to find areas most in need and maximize the impact of their programs. Collecting data more frequently would enable mass B-12 deficiency to be more visible and result in direct program action.

Mechanism by which these outcomes will be achieved

Currently, to assess the status of Nigeria, it costs $10 million USD which allows for surveys to be conducted every 20 years. However, with a biosensor (<5$ per assay), we can shrink the costs of national surveys by 40x ($250,000 USD), enabling actionable data to be collected every 2 years. This cost would be achieved via leveraging existing resources like paper and off-the-shelf glucometers. A fully automated POC biosensor would avoid the need to ship blood samples overseas to first-world labs (eliminating multi-million dollar cold supply chains using expensive lab equipment), and enable rapid door-door testing using finger prick blood. This data would be used by nonprofit data collectors, which they would use to make judgments on whether an intervention program is needed in the region and to what scale. Ultimately, B-12 intervention would be able to reach those directly in need.

Most important indicators the project is succeeding

1. The development of a reliable and low-cost (<$5 each) test fully validated and approved
    a. Results have an <10% deviation from the gold standard lab test
    b. Results have an <10% deviation when used in different regions of Nigeria, considering varying environmental conditions
    c. Workers of limited training are capable of producing accurate results when administering the test due to the design of the biosensor
2. Introduction of intervention programs based on mass screening for B12 deficiency in Nigeria, or other LMICs

Why we are well suited to implement this project

Ashley Mo (project lead) and Liesl Anggijono will be working on the project. Both of us have been researching extensively in the diagnostic space over the last year, including POC biosensors for both first and third world applications. We are considering adding a third person in September (likely a biomedical or chemical engineering student from MIT or Purdue) who will help Ashley on the technical side.

In February, we arranged a series of 1-1 chats with a dozen data collectors from seven nonprofits/NGOs (WHO, CDC, UNICEF, Asia's Food Fortification Program, Micronutrient Forum, PATH, USAID) who have all validated the problem that high cost = lack of B-12 data = lack of program action. All mentioned a low-cost biosensor, especially for vitamin B-12 (an overlooked nutrient) "would be a dream." We have worked over the last two months to map out a solid design of the biosensor + reaction mechanisms. Now, we are compiling a list of

materials and writing out the building procedures. On the technical side, there has been much positive feedback on the design from scientists at Harvard, MIT, and Purdue University, who are currently helping to provide more feedback and guidance related to prototyping. We have contacts with lab spaces at Purdue and MIT, and we will confirm what facility we will use by the end of September. We are looking to build our first wet-lab prototype this November or December.

If the project gets funded, but doesn't achieve the desired outcomes

The biggest challenge will be to make sure the assay works under field conditions. We believe this can be ensured in the lab; however, fluctuating temperatures and pH may result in high error rates that can make nonprofits/governments reluctant to collect data with the device.

Similar projects, and why ours represents an improvement

There have been other lower-cost POC biosensors developed to detect vitamin B-12. However, their biggest flaw is that they measure total B-12 (the raw protein itself). Only B-12 attached to the transporter protein transcobalamin is taken up by cells for use, with 75% of the circulating protein being biologically inert. Thus, measuring total B-12 does not give an accurate picture of one's B-12 status. This can result in false negatives since an individual can have normal total B-12 levels, but a smaller fraction may be absorbed by the body, thereby making them deficient. As a result, it has been recommended to measure B12 bound to transcobalamin (TC), also named holotranscobalamin (holoTC). This is what our device targets.

One of the most broadly used assays for holoTC is the active B-12 assay from Axis-Shield. Their assay with 96 wells (96 tests) costs $1,286.40 USD which puts each test at ~$13. This does not meet the <$5/assay goal. Because it is a traditional ELISA test, it is not a POC test, Rather, it is a four-step process that involves washing and incubation, and must be performed by trained lab personnel. The test must also be conducted in a controlled lab facility, making it not suitable to employ in tents or homes in low-resource regions. Thus, samples would still have to be shipped overseas. There are also holo-TC assays by Siemens Healthineers, however, they also use an ELISA.

Largest risks & uncertainties (+ how we will address them)

The greatest concern is that the assay has a greater than 20% error rate in accurately measuring B-12 concentrations. This is a big enough error rate that a severely deficient population could be measured as perfectly healthy and vice versa. If B-12 deficiency is estimated to be greater than the actual severity, unnecessary intervention may be put in place, wasting millions of dollars. In contrast, if B-12 deficiency is estimated to be less than the actual severity, the deficiency may not be addressed fully or at all. If the assay is found to have this great of a deviation, it will have to be further optimized which in turn may prolong time to implementation.

In addressing this risk, the assay developed in the lab would have to be thoroughly validated against gold standard assays (<10% deviation as recommended by nonprofit/governmental data collectors). Since we are measuring holoTC, our gold standard assay is the active-B12 immunoassay from Axis-Shield, which has received US Food and Drug Administration approval. The test itself would later also have to undergo FDA approval, as well as IRB or ASIA PAC approval depending on if we deploy the assay in Africa or Asia. Predicting development time is hard, but we will simply have to secure funding whenever we need to as fast as possible, garner as much support from experts in the technical field who could help us speed up lab processes, and/or use automated equipment.

How this grant could be actively harmful

There doesn't seem to be any predictable harm, since we will just be using the grant to build a wet-lab prototype. Since it will just be our prototype, it will not be implemented for data collection just yet, and we will ensure that it is thoroughly validated against gold standard B-12 assays before we ask for approval from federal organizations (both in the US as well as the country/countries the assay will be deployed). We do not see any potential damage to the reputation of funders or the EA community.

What will it look like if this project has gone well in 12 months' time?

In 12 months, we are aiming to have a working biosensor prototype in the lab.
This involves multiple checks:

- Completing each portion of the building protocol (outline of 5 major steps)
- Achieving a level of specificity greater than 90% (performing cross-reactivity using a sample with holoTC and TC mixed in with other B-12 related proteins like haptocorrin and intrinsic factor)
- Achieving a level of sensitivity greater than 90%, and confirming the limits of detection we initially calculated
- Comparing the performance of the assay (both sensitivity + specificity) with the holoTC Axis-Shield Assay, and seeing a deviation that is no larger than 10%

Ultimately, the grant would be able to make this "prototyping process" possible.

Which countries will you operate from, and in which countries will you implement projects?

1) The prototype will most likely either be built in Massachusetts, Indiana or the Bay area (definitely in the US).
2) We plan to test the assay in Nigeria, but depending on NGOs involved we will adjust. There are a large number of potential countries, with Tanzania and Nepal being recommended as well. The main issue is to test the assay in an area with suspected B-12 insufficiency. This will be fully determined after we have built and validated the biosensor.

Project Specific Questions

Why not a biosensor for ferritin? Why B-12?
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7374411/)

Ferritin was one of our first choices until we found other low-cost ferritin assays designed specifically for low-resource areas. For instance, PATH has developed the Q-Plex™ Human Micronutrient (7-Plex), which is a multiplex immunoassay for inflammation, iron, vitamin A, iodine, and malaria. They are utilizing ferritin as a biomarker for iron. The assay enables each analyte (including ferritin) to be analyzed for $1.43 USD and is specifically designed to be used by public health researchers working in remote regions of low-middle income countries. We also recently chatted with a group of graduate students from MIT who are making a low-cost paper-based assay for ferritin which they are aiming to implement in low-resource regions. It's also worth mentioning another paper on a paper-based electrochemical biosensor for ferritin, which so happens to be the exact "biosensor format" we would have made due to its ability to perform low-cost quantification.

Iron intervention ranks as one of the top three most common micronutrient programs. For example, Nutritional International, one of the largest nonprofits dedicated to mitigating micronutrient deficiency, alone has already established seven national iron supplementation programs. We want to focus on micronutrients that don't have many known programs established. We couldn't find a single vitamin B-12 fortification program from Nutritional International, which is a huge problem since vitamin B-12 deficiency is just as dangerous as iron deficiency. Permanent neurological damage arises which leads to loss of function of limbs and vision.

What is the evidence that governments or other groups would be willing to respond if they discovered that large proportions of their population are B12 deficient?

There have been numerous occasions when the collection of micronutrient data directly led to the establishment of nationwide fortification/supplementation programs - namely for vitamin A, iodine, and folate. We believe the same matter is reflected with B-12: if it is found that large proportions are found to be B-12 deficient, then governments and/or nonprofits would be willing to respond.

Case study 1: When vitamin A was first identified as a public health concern in Central America, the Institute of Nutrition of Central America and Panama (INCAP) directed its efforts to overcome this problem and developed the technology to fortify sugar with vitamin A. Fortification programs were then implemented in several countries across the region after closer evaluation was conducted and large populations were found to be deficient. As a notable example, a sugar fortification program was implemented in Guatemala in 1975 (still existing today), with the support of INCAP and the Government of Guatemala, through the Ministry of Health in response to a detected population vitamin A deficiency. Populations of

children in Guatemala are nearly eradicated of vitamin B-12 deficiency today (less than 2% prevalence of serum retinol < 20 µg/dL).

Case study 2: The availability of info on iodine has provided multiple countries with the rationale to initiate salt iodization programs to combat iodine deficiency. Before the 1970s, iodine deficiency disorder (IDD) was never recognized as a problem in Nepal until a nationwide survey was conducted. Three years later, the government established the first national IDD control program in response, with salt being iodized and iodized oil capsules being distributed to 15 remote districts. Less than a decade later, 90% of households were eliminated from deficiency.

Case study 3: Data on red blood cell folate status from the US National Health and Nutrition Examination Survey have allowed the CDC to identify population groups with sub-optimal folate concentrations who need to be targeted with additional interventions beyond wheat flour fortification (to prevent neural-tube defect-affected pregnancies).

Could you please explain what convinces you that you can create a low-cost point-of-care biosensor that is superior to what can be purchased?

As mentioned earlier, all of the existing POC B-12 tests are designed to measure total B-12 (both active + inactive), which does not provide an accurate reflection of B-12 status. This is also the biomarker used in B-12 lab tests for current data collection. Only about ¼ of raw B-12 is bound to transcobalamin (TC), and only B-12 bound to it is taken up by cells for use. Measuring total B-12 is problematic since an individual may have seemingly normal levels; however, a smaller amount than normal may be attached to TC, reflecting a lower amount of active B-12 that is used by the body. It is recommended by The Biomarkers of Nutrition for Development (BOND) project to use holoTC, the complex of B-12 and TC, to assess B-12 levels instead.

Our biosensor will measure holoTC. Also answered in a question above, the only holoTC assays that exist are ELISAs by Axis-Shield and Siemens Healthineers, which are multi-step processes, require trained lab workers, and must be conducted in clean lab facilities thereby making their implementation in low-resource settings (in tents, homes, etc.) not practical. Analyzing samples using ELISAs would not solve the problem of having to ship them overseas and eliminate the costs of the cold supply chain, storage equipment, etc. ELISAs are also not the cheapest, costing ~$13 USD per test, which does not reach our goal of <$5 per assay.

Not only will the biosensor measure holoTC, but it will be the first low-cost version. We will achieve the <$5 USD cost per assay by piggybacking on existing resources like paper, off-the-shelf glucose strips, and glucometers, eliminating the need to customize our electrodes and spend $400 on each potentiostat (which is the case with current B-12 biosensors). Paper not only costs less than 1 cent, but we can use it to develop an assay that remodels a drug-store pregnancy test (aka a lateral flow), thereby enabling the assay to be automated and only requiring the sample and substrate to be dropped into the well, which is a task that workers of limited experience can perform.

Evidence the project can be completed well

How we are already on the way to achieving our goals with this project

First, we determined the need for a B-12 biosensor. In February, we had 1-1 Zooms with a dozen data collectors from nonprofits like the WHO, CDC, Micronutrient Forum, PATH, etc, where we validated that one of the largest barriers to mitigating micronutrient deficiency is being able to assess thousands of blood samples data at low-costs. We have also gotten several recommendations from them to focus into vitamin B-12 first, due to the need for a biosensor that measures a better biomarker other than total B-12, and since most efforts into the development of low-cost assays have been already focused towards ferritin, iodine, hemoglobin, and vitamin A.

Afterwards, we thoroughly mapped out the biosensor design:

- Thus far, the design has gotten positive feedback from 10 profs, postdocs, and grad students from MIT, Harvard, Purdue, and Johns Hopkins who work specifically with biosensors
    - Various aspects of our design were made based on recommendations from profs (i.e. the glucometer aspect was from Dr. Ariel Furst from MIT)
- We've received consistent guidance from Ebba Nexo, who works specifically with B-12 assays, to map the best method of assessing holoTC (including which specific antibodies to use) which we've incorporated into our design
- We've theoretically calculated that the biosensor should be able to detect holoTC concentrations (100 pmol/L) within 2.5 minutes, showing potential in our design in that it can achieve high levels of accuracy with existing resources)
    - Chemistry calculations
- Regarding the feasibility of prototyping, the experts we asked replied that is possible by taking it step by step (which is mapped out in our building protocol)
- We also asked them about the feasibility of implementing design in low-resource regions, and they said that paper and repurposing $20 glucometers would certainly make assays under $5 possible

At the moment we have progressed to the stage where we need to physically build version 1.0 of this biosensor. We're in the process of refining the building protocol, with support from scientists + profs, which we plan to have solidified by October. We've also made a detailed list of the exact materials we need to purchase, including how much of each we need. The spreadsheet is attached in the budget section below.

Past projects

Ashley:
Related to other projects I've been working on in the global health & diagnostics space, I started Project LungTech. The project was partnered with MIT's Mobile Tech Lab, where we

built a prototype algorithm that could use machine learning to diagnose asthma, COPD, Covid-19, and healthy subjects via a cough recording. I spoke about the idea at IBM's Cascon and Evoke Conference, at the Johns Hopkins Global Health Leaders Conference, and the research is being published at IEEE's Global Health and Technology conference. We partnered with the OKB Hope Foundation and are working to implement the algorithm at their clinics in rural Ghana.

However, since the summer, most of my time and energy has been directed towards Project Helio, and I believe great progress will be seen similar to Project LungTech. But that will only be possible if we have the funds to purchase prototyping materials.

Before February this year when we turned to micronutrients, project Helio was focused on making a remote POC lupus monitoring system. Although my former teammate and I realized the concept of the biosensor ultimately did not make sense after talking with a dozen rheumatologists, we did map out a thorough design of the biosensor and understood the required electrochemistry.

For nearly a year before Helio, I researched various POC diagnostics for Covid-19, from lateral flows to PCR tests in a box. I wrote a review article last year summarizing my findings.

Liesl:
Liesl has previously worked on designing a microfluidic for the rapid diagnosis of endometriosis, a reproductive disorder.

Budget + Funds

The plan if we received $30,000 USD six week from now

Within the first month, ⅓ of the grant would be used to purchase materials to create the conjugate pad of the lateral flow. This involves creating antibody-enzyme and antibody-nanoparticle conjugates. We would also be testing five different clones of antibodies to determine the best pair for holoTC (we are planning to use a sandwich format). It would take roughly one month for all reagents needed to be shipped over. During that time we would be continuing to refine the building protocol, and confirm our lab space. We would aim to begin lab work around mid-December 2022 and estimate this first step would take 2-3 weeks.

Since a sample cannot be directly applied to the lateral flow test (shown in image one of mockups), we would need to create the B-12 coated magnetic beads which will cost $5,000 USD. In late January orders would be placed to purchase raw B-12 proteins and magnetic beads. We would aim to perform this process around late February and estimate it would take another week.

Around early March, we would spend another $5,000 USD to assemble the lateral flow using the components created for the conjugate pad and the lateral flow test kit. The lateral flow assembly process would take place in the lab during two weeks in April.

Afterward, we would begin to test the assembled biosensor for cross-reactivity and compare its sensitivity and specificity with the Axis-Shield holoTC assay. Materials would be purchased in late April, and this would be done in late May. We would aim to have a >75% sensitivity and specificity, with a <20% deviation. As we refine sections of the assay after those six months we would strive to reach >90% and <10%, respectively.

The remainder of our funds would be set aside to purchase more antibodies, nanoparticles, enzymes, and biomarker samples since we do not know how many errors we would be making along the way. It is likely lab work would have to be repeated for some sections.

How much funding we are requesting

$30,000 USD

What we would do with the amount specified

Purchase wet-lab prototyping materials: lateral flow test kits, glucometers, glucose strips, antibodies, gold nanoparticles, buffer solution, magnetic beads, holoTC (we are still trying to find pure form), human serum, and the Axis-Shield assay.

Spreadsheet of all the materials we are planning to purchase, including their costs and quantities.

What we would be most likely to do if our project is not funded.

The lack of funding is the only barrier at the moment towards making progress since we are finishing everything that we will need to be written or mapped out virtually in the coming month. Wet-lab bio is also so expensive it cannot be self-funded. Thus we would most likely continue to apply for further grant opportunities.

Other funding we are receiving (even if it is not related to this project)

This project has received $1,000 USD from a private donor who is not a part of an established organization (she was giving out $1k grants). We stated these funds would contribute to our savings to purchase materials.

Ashley Mo has received $1,000 USD for Project LungTech from 1517 Funds (Medici Project) to collect more cough data.

Other grant application submitted for this project

We previously submitted to Emergent Ventures, however, we are still waiting on a response (it has been quite a long time, and we are unsure if they have been giving out more grants since 2021).

Liesl submitted a grant application to the Masason Foundation. The application has been pending for a while as well and we are also unsure when we will hear back soon if at all.